

EXHIBIT

Γ 1: Int J Androl. 1997 Feb;20(1):45-54. Links

Improved preservation of ultrastructural morphology in human spermatozoa using betaine in the primary fixative.

Swan MA.

Department of Anatomy and Histology, University of Sydney, NSW, Australia.

Improved preservation of the ultrastructural morphology of human spermatozoa was obtained by filtration onto a Millipore filter (0.45 micron pore size) followed by fixation for 1 h in a fixative containing 3% glutaraldehyde, 1% formaldehyde and 21 mM betaine in Hepes or cacodylate buffer. Betaine raised fixative osmolality only slightly but markedly improved the ultrastructural appearance of the spermatozoa; it is known to function as an osmoprotectant in other situations, and may protect the cells from osmotic damage during the initial stages of fixation. The method is suitable for oligozoospermic samples as they are concentrated when the seminal plasma passes through the filter. Utilizing fixation after swim-up, it is suitable also for highly viscous semen. The fixative gave equally good preservation of sperm heads and tails and can be stored prior to use. The Millipore filter did not dissolve until placed in acetone prior to embedding, simplifying the exchange of solutions during processing. Improved preservation of rat epididymal ultrastructure was obtained by addition of betaine to the fixative of Ito & Karnovsky (1968), using immersion fixation.

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